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Keywords: Soil ingestion, potentially toxic element, bioaccessibility, contaminated land, human health risk, soil particle size

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Editor

Chemosphere

29 March 2016

Dear Editor,

I submit a manuscript entitled "**Particle Size Effects on Bioaccessible Amounts of Ingestible Soil-borne Toxic Elements**" for possible publication in your journal.

Thank you in advance for your consideration.

Yours sincerely,

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Highlights

- Ingestion of contaminated soils and soil-derived dusts poses a risk to human health
- Bioaccessible amounts of 9 elements in multi-contaminated soils were determined
- Soil particle size significantly affected bioaccessible amounts of potentially toxic elements
- It also had significant effects on bioaccessibility of As and Al in intestinal environment
- Further division of the <0.25 mm soil fraction is necessary for better health risk assessment

*Response to reviewers/editor in question & answer format (word file)
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Authors Responses to Comments from Editor and Reviewers

Dear Editor,

Thank you for your invitation to submit a revised manuscript for further consideration. We would also like to thank the reviewers for their constructive comments and suggestions.

We have now made changes to address the reviewers' comments and the point-by-point responses to their comments are provided below.

Reviewer #1

Reviewer's Comment

This is a well designed and conducted experiment, with clear objectives, procedures and outcomes. It deals with an important topic that has not been explored thoroughly.

Authors' Reply

We appreciate the reviewer's comments

Suggestions on the text

Reviewer's Comment

Line 68, insert "unified bioaccessibility method" before UBM.

Authors' Reply

This has now been done, as suggested by the reviewer (highlighted in blue in Page 4, Line 72).

Reviewer's Comment

Line 75, give more information about the selection of samples FG1 and FG2

Authors' Reply

We have now added relevant information, as per the reviewer's suggestion (highlighted in blue in Page 4, Lines 79-86).

Reviewer's Comment

Line 229, "was also very much higher".

Authors' Reply

Change has been made following the reviewer's suggestion (highlighted in blue in Page 9, Line 206).

Reviewer's Comment

Line 249, "proportion of more soluble Al species".

Authors' Reply

Correction has been made (change "sore" to "more") (highlighted in blue in Page 10, Line 227).

Reviewer #2

Reviewer's Comment

In Introduction, line 55, please provide reference for the statement that heavy metal concentrations tend to be higher in the finer fractions.

Authors' Reply

The following two references have been added to support the statement that heavy metal concentrations tend to be higher in the finer fractions (Page 3, 57-58 and the reference list in the revised manuscript):

Li, Q., Ji, H.B., Qin, F., Tang, L., Guo, X.Y., Feng, J.G., 2014. Sources and the distribution of heavy metals in the particle size of soil polluted by gold mining upstream of Miyun Reservoir, Beijing: implications for assessing the potential risks. Environ. Monit. Asses. 186, 6605-6626.

Zong, Y.T., Xiao, Q., Lu, S.G., 2016. Distribution, bioavailability, and leachability of heavy metals in soil particle size fractions of urban soils (northeastern China). Environ. Sci. Pollut. Res. DOI 10.1007/s11356-016-6652-y.

Materials and Methods

Reviewer's Comment

Table 1.: Please provide data on organic carbon and clay content in soils used for experiments.

Lines 81-89.: Please provide information on percentage share of different soil fractions in total soil mass for both soil samples.

Authors' Reply

Information on organic carbon content and the percentage share of different soil particle fractions has now been provided in Table 1 (highlighted in blue) and stated in the text (highlighted in blue in Page 5, Lines 95-99).

*Revised manuscript with changes marked
Click here to view linked References

Particle Size Effects on Bioaccessible Amounts of Ingestible Soil-borne Toxic Elements

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Abstract

The unified BARGE method was used to examine the effects of soil particle size on the bioaccessible amounts of potentially toxic elements in multi-contaminated soils from a closed landfill site. The results show that bioaccessible As, Al, Cd, Cr, Cu, Mn, Ni, Pb and Zn increased with decreasing soil particle size and the <0.002 mm soil fraction contained much greater amounts of the bioaccessible elements, as compared to other soil fractions (0.002-0.063 mm, 0.063-0.125 mm, and 0.125-0.250 mm). As, Al and Cr had much lower bioaccessibility, as compared to the six cationic heavy metals. In contrast with other elements, As bioaccessibility tended to be higher in the gastrointestinal phase than in the gastric phase. There was a significant soil particle size effect on bioaccessibility of As and Al in the gastrointestinal phase: As bioaccessibility decreased with decreasing particle size, and the finer soil fractions tended to have a higher Al bioaccessibility, as compared to the coarser soil fractions. The research findings prompt the need for further division of soil particle size fractions in order to more accurately assess the bioaccessible amounts of soil-borne potentially toxic elements in contaminated lands.

Keywords: Soil ingestion, potentially toxic element, bioaccessibility, contaminated land, human health risk, soil particle size

1 Introduction

In vitro methods to simulate dissolution of soil-borne potentially toxic elements (SBPTEs) in human gastrointestinal tract have gained increasing acceptance as a routine protocol for assessing the human health risk from ingesting SBPTEs (Oomen et al., 2002; Li et al., 2015). The unified bioaccessibility method (UBM) developed by the Bioaccessibility Research Group of Europe (BARGE) recommended the use of <250 µm soil fraction for assessment of bioaccessibility of SBPTEs (Wragg et al., 2011; Denys et al., 2012; Collins et al., 2015). This assumes that all soil particles with a diameter less than 250 µm are proportionally ingested by human during a given period of time. However, it is highly likely that finer soil particles tend to be more ingestible, as compared to coarser soil particles. For example, Ikegami et al. (2014) found that over 90% of soil particles adhered to children's hands were <100 µm in diameter. Ingestible dust particles derived from soils could be much finer than dermally adhered soil particles (Mahowald et al., 2014). Since heavy metal concentrations tend to be higher in the finer fraction than in the coarser fraction (Li et al., 2014; Zong et al., 2016), the standard method using <250 µm soil fraction has its limitations when being used to assess human health risk associated with soil ingestion by children or ingestion of soil-derived dusts in the areas adjacent to contaminated lands (Martin et al., 2015). In addition, most of the work reported so far focused on a limited number of SBPTEs, especially lead and arsenic (Basta et al., 2007; Denys et al., 2007; Palumbo-Roe and Klinck, 2007; Bosso and Enzweiler, 2008; Denys et al., 2009; Broadway et al., 2010; Roussel et al., 2010; Lu et al., 2011; Mingot et al., 2011; Appleton et al., 2012). For soils contaminated with multiple SBPTEs, the damaging effects of ingesting such soil materials on human health could be much more severe due to combined toxicity of various SBPTEs. It is therefore important to understand the simultaneous responses of different SBPTEs to chemical attack from gastrointestinal fluids in order to better assess the combined toxic effects of multiple SBPTEs on human beings exposed to different sizes of ingestible soil/soil-derived dust particles.

In this study, four further-divided fractions of <250 μm soil fraction were used to examine the effects of soil particle size on extracting a range of potentially toxic elements from contaminated soils using the unified bioaccessibility method (UBM) in vitro gastrointestinal tract method. The objective was to determine whether further particle size division is necessary in order to more accurately assessing the human health risk from ingestion of multi-contaminated soil/soil dust particles originated from contaminated lands.

2 Materials and Methods

2.1 The soil materials

Two multi-contaminated soil materials were selected for this study following a soil screening test to identify appropriate soil samples from 27 locations within a closed landfill site adjacent to a residential area in the Greater Manchester region, England. This site received substantial amounts of industrial wastes during the period of Industrial Revolution. The soil samples were collected from the surface soil layer (0-10 cm), which is a readily available source of contaminated soil materials for human ingestion. The soil samples chosen contained a wide range of multiple SBPTEs at elevated concentrations with Sample FG 1 having a slightly alkaline pH and Sample FG2 having a slightly acidic pH (Table 1).

After collection, the soil materials were oven-dried at 40 °C and then crushed to pass a series of sieves (2.00, 0.25, 0.125 and 0.063 mm) to obtain the following soil particle fractions: 0.25-2.00 mm, 0.125-0.250 mm, 0.063-0.125 mm, and <0.063 mm. The <0.063 mm fraction was then suspended in deionized water and stirred by a magnetic stirrer for 1 h. The dispersed soil suspension was placed in a 1 L cylinder and stood for 24 h to allow the >0.002 mm soil particles to settle (McCarty et al., 2016). The remaining suspended materials were then separated from the settled soil materials to obtain two further soil particle fractions: <0.002 mm and 0.002-0.063 mm. The 0.25-2.00 mm

fraction was not used in this experiment. The four soil fractions tested in this study therefore include: <0.002 mm, 0.002-0.063 mm, 0.063-0.125 mm and 0.125-0.250 mm. The percentage share of these different soil fractions in the <0.250 mm soil mass was 1% (<0.002 mm fraction), 13% (0.002-0.063 mm fraction), 32% (0.063-0.125 mm fraction) and 54% (0.125-0.250 mm fraction) for Sample FG1, and 1% (<0.002 mm fraction), 20% (0.002-0.063 mm fraction), 32% (0.063-0.125 mm fraction) and 47% (0.125-0.250 mm fraction) for Sample FG2 (Table 1).

2.2 Element extraction procedure

The unified BARGE method (Denys et al., 2012) was slightly modified and used for extraction of a range of elements in the soil samples. Briefly, for the gastric phase extraction, 9.0 mL of synthetic saliva was added to 0.6 g of each soil sample in a 50 mL centrifuge tube. After shaking by hand for 10 seconds, 13.5 mL of synthetic gastric fluid was added to the tube. The pH of the suspension was adjusted to 1.20 ± 0.05 with HCl or NaOH. The capped tube was placed in a water bath and the content was incubated at 37 °C for 1 h. After incubation, the gastric phase sample was centrifuged for 15 min at 4500 rpm. The supernatant was then filtered, acidified with 0.5 mL concentrated HNO_3, and stored at 4 °C prior to analysis. For the gastrointestinal phase extraction, a subsample after gastric phase extraction described above was further treated with 9 mL of synthetic bile and 27 mL of synthetic duodenal fluids. The pH of the extracting solution was adjusted to 6.3 ± 0.5. The sample was then placed in a water bath and the content was incubated at 37 ºC for a further 4 h. After incubation, the gastrointestinal phase sample was centrifuged for 15 min at 4500 rpm. The supernatant was then filtered, acidified with 1.0 mL concentrated HNO_3 and stored at 4 °C prior to analysis.

2.3 Analytical methods

The total concentration of various elements in different soil particle fractions was determined by ICP-OES (Agilent Varian 720-ES) after HNO_3-HCl-H_2O_2 digestion in a microwave digester

(CEM Corporation MARS 5 Digestion Microwave System). Element concentration in the gastric and gastrointestinal phase extracts was also measured by ICP-OES. pH in various soil-fluid mixtures was measured using a calibrated pH meter (JENWAY-3510).

2.4 Statistical analysis methods

Significant difference analysis was conducted using SPSS (version 17.0). The experimental data were analyzed by one-way analysis of variance (ANOVA) and the means compared using significant difference (Duncan) method at 5% level. The relationships between the total concentration of various elements and that extracted by the simulated gastric or gastrointestinal fluids for different soil particle fractions were determined by linear regression.

2.5 Quality control and quality assurance

All the chemicals used in the experiment were of analytical grade. Ultrapure water was used throughout the entire experiment. The experiment was performed in triplicate and all the samples were run in a single batch to allow reasonable comparison.

The relative standard deviation (RSD) ranged from 4.02 to 8.72% for the gastric phase and from 4.21 to 9.15% for the intestinal phase, indicating a high level of agreement between replicates of the experiment runs. Since no appropriate certified reference materials (CRM) with comparable soil particle size fractions and the required range of contaminants are available at the time of this work, no CRM was used in this study. While the accuracy of the data was not confirmed, the high repeatability of replicated experiment ensure that the data are sufficiently precise for comparison between the different soil particle fractions. Therefore, the experimental data is of satisfactory quality for this work aiming at examining the effects of soil particle size on mobilization of SBPTEs in simulated gastrointestinal tract environments.

3 Results

3.1 Total element concentration

The total concentration of various elements estimated by HNO_3-HCl-H_2O_2 digestion method is given in Table 2. In general, there was a clear trend that the concentration of each element significantly increased with decreasing soil particle size. Except for Cr and As in FG2, the concentration of any elements for the <0.002 mm fraction was at least 1 time higher than that for the 0.002-0.063 mm fraction.

3.2 Elements by the gastric phase extraction

There was a clear trend that the amount of elements extracted by the synthetic gastric fluid increased with decreasing particle size for all the investigated elements in all the two samples (Table 3). The <0.002 mm fraction had the absolutely highest concentration in all cases with a value at least 1 time higher than that of any other soil particle fractions. In FG1, there was no significant difference in concentration between the 0.063-0.125 mm fraction and the 0.125-0.25 mm fraction for Al, Cr, Fe,Ni, Pb and Zn. In FG2, there was no significant difference in concentration between the 0.002-0.063 mm fraction and the 0.063-0.125 mm fraction for Al, As, Cu, Fe, Mn, Pb and Zn.

3.3 Elements by the gastrointestinal phase extraction

A similar trend to the gastric phase extraction was observed for the gastrointestinal phase (Table 4). The amount of elements extracted tended to increase with decreasing particle size for all the investigated elements in all the two samples. The <0.002 mm fraction had the absolutely highest concentration in all cases though the value gap between the <0.002 mm fraction and other fractions was not as large as those in the gastric phase. In FG1, there was no significant difference in concentration between the 0.063-0.125 mm fraction and the 0.125-0.25 mm fraction for the amount of elements, except for Cu and Mn. In FG2, there was no significant difference in As between the

164 0.002-0.063 mm fraction and the 0.063-0.125 mm fraction, and Cr between the 0.063-0.125 mm
165 fraction and the 0.125-0.25 mm fraction.

166

167 **4 Discussion**

168 There was a close relationship between the total concentration and either gastric phase or
169 gastrointestinal phase for all the investigated elements (Table 5). This indicates the strong control of
170 total amount of an element on the mobilizable amounts of that element in the gastrointestinal tract.
171 Therefore, the observed trend that the bioaccessible amount of either gastric phase or gastrointestinal
172 phase increased with decreasing soil particle size can be largely attributed to the increase in the total
173 element concentration with decreasing soil particle size. In terms of bioaccessibility (the percentage
174 of bioaccessible fraction in the total element pool), the particle size effect was more complex for the
175 investigated elements (Fig. 1 and Fig. 2).

176 The bioaccessibility of the investigated elements varied from element to element. For arsenic,
177 the bioaccessibility for the gastric and intestinal phases were less than 7% and 13% (Fig. 1b and Fig.
178 2b), respectively. The relatively higher bioaccesibility of As in gastrointestinal phase in spite of
179 higher pH in the extracting solution suggests that mobilization of soil-borne As in the gastrointestinal
180 tract was not largely controlled by the fluid acidity. During the gastric phase extraction, As bound to
181 iron compounds might be mobilized due to dissolution of these iron compounds under acidic
182 conditions. However, the dissolution rate of iron compounds tended to be very small with only <1%
183 of the total soil Fe being dissolved in the simulated gastric fluid for most of the soil particle fractions
184 (refer to Tables 2 and Table 3). This low solubility of iron compounds could limit the release of As
185 from the soil. During the intestinal phase extraction, the introduction of bicarbonate could enhance
186 the liberation of As from the soil under circumneutral pH conditions (Saalfield and Bostick, 2010).
187 There was no statistically significant difference in gastric phase of As among the four different

188 particle fractions. However, the bioaccessibility of the gastrointestinal As phase decreased
189 significantly with decreasing particle size, suggesting that As species held by the coarser soil
190 particles tended to be mobilized easily in the intestinal environment. This may be attributed to
191 weaker affinity between the As species and the binding site on the coarser soil particles. Li et al.
192 (2015) also observed higher As bioaccessibility for gastrointestinal phase, relative to gastric phase in
193 some As-contaminated soils.

194 Another oxyanion-forming element, Cr, also had very low bioaccessibility (<4%) in both the
195 simulated gastric and gastrointestinal environments (Fig. 1d and Fig. 2d). However, unlike As, Cr
196 bioaccessibility tended to be higher under the simulated gastric conditions than under the simulated
197 gastrointestinal conditions, suggesting that the acidity of digestive fluid plays a certain role in
198 mobilizing Cr in gastrointestinal tract. The results obtained from this study generally agreed with the
199 work by Broadway et al. (2010) who reported a Cr bioaccessibility of 5% in some Glasgow soils and
200 the bioaccessibility of Cr was greater in the gastric phase than in the gastrointestinal phase by a
201 factor of 1.5.

202 In contrast with oxyanion-forming elements, all other metals investigated in this study showed
203 much higher bioaccessibility in the simulated gastric environment (>30% except for Ni; refer to Fig.
204 1c, Fig. 1e-i, Fig. 2c and Fig. 2e-i). This suggests that most of soil-borne heavy metals were highly
205 soluble in the simulated gastric fluid. However, it is interesting to note that the bioaccessibility of
206 these metals was also very much higher in the simulated gastrointestinal environment where the fluid
207 pH was raised to 6.3. This can be attributed to the presence of digestive enzymes and bile in the
208 simulated intestinal fluid (Oomen et al., 2004; Li et al., 2013). Bile is capable of forming soluble
209 metal complexes under non-acidic pH conditions (Feroci et al., 1995; Oomen et al., 2003). Within
210 these metals, Cu exhibited an unusually high bioaccessibility (>35% for FG1 and >25% for FG2).
211 This was in agreement with work by Li et al. (2013) who found that Cu had much higher
212 bioaccessibility, as compared to other divalent heavy metals.

213 The relatively low bioaccessibility of Pb may be attributed to its low solubility under high pH
214 conditions. Close examination found that for Cd, Cu, Pb and Zn in FG1, the bioaccessibility tended
215 to be higher in the coarser fraction than in the finer fractions, suggesting that for certain soil
216 materials, a higher proportion of these metals that are bound to coarser particles may be liberated
217 from the surfaces of these soil particles.

218 Aluminium (Al) bioaccessibility ranged from 5.9 to 13% for the gastric phase. However, it
219 markedly decreased to less than 3% for the gastrointestinal phase (Fig. 1a and Fig. 2a). This reflected
220 re-immobilization of Al upon change in pH from 1.2 under gastric condition to 6.3 under intestinal
221 condition. Shock et al. (2007) investigated the bioaccessibility of Al in the soils affected by mine
222 dust deposition and found a range of 0.31−4.0% for Al bioaccessibility in the gastric phase. They
223 concluded that the low value of Al bioaccessibility in their samples was consistent with the
224 predominance of Al in relatively insoluble aluminosilicate minerals. Reis et al. (2014) also reported a
225 level of gastric phase Al bioaccessibility similar to that of Shock et al. (2007) for some French soils
226 and dusts. The relatively higher gastric phase Al bioaccessibility of soils in this study appears to
227 suggest that there was a higher proportion of more soluble Al species present in the investigated
228 soils. It is interesting to note that the gastrointestinal phase Al bioaccessibility tended to be higher in
229 the finer fractions (<0.002 mm and 0.002-0.063 mm) than in the coarser fractions (0.063-0.125 mm
230 and 0.125-0.250 mm). The reasons for this are not clear. Probably, the finer fractions contained more
231 organically complexed Al species, which could stay soluble under circumneutral pH conditions
232 (Driscoll and Letterman, 1988) such as that in the simulated intestinal environment.

233 The research findings obtained from this study have implications for assessing health risk
234 associated with human exposure to ingestible contaminated soils. For more accurate evaluation of
235 human health risk from contaminated soil/soil-originated dusts, further division of finer soil fractions
236 from the standard <0.25 mm fraction is needed. For the clay fraction (<0.002 mm), significantly
237 larger amounts of elements are mobilized in the simulated gastric and intestinal environments. This

increased amount of bioaccessible SBPTEs is particularly relevant to assessing human health risk from exposure to contaminated soils in areas experiencing frequent strong wind. Previous research demonstrated that the heavy metals could move with wind along the wind direction and the soil samples had higher heavy metal concentrations at the downwind direction (Chen et al., 2010). While Al is viewed as a potentially toxic element (Krewski et al., 2007), bioaccessibility assessment of soil-borne Al is rare. The significantly elevated gastrointestinal phase Al bioaccessibility in the finer soil fractions discovered in this study is important since bioaccessible Al tends to be primarily adsorbed in the distal intestine (Krewski et al., 2007).

5 Conclusion

The size of soil particles had significant effects on the bioaccessible elements of potential toxicity. There was a clear trend showing that As, Al, Cd, Cr, Cu, Mn, Ni, Pb and Zn increased with decreasing soil particle size and the <0.002 mm soil fraction contained much greater amounts of the bioaccessible elements, as compared to the coarser soil fractions investigated in this study. Cd, Cu, Mn, Ni, Pb and Zn tended to have much greater bioaccessibility than did As, Al and Cr. As bioaccessibility tended to be higher in the gastrointestinal phase than in the gastric phase. There was a significant soil particle size effect on bioaccessibility of As and Al in the gastrointestinal phase. As bioaccessibility decreased with decreasing particle size. But Al bioaccessibility tended to be higher in the finer soil fractions than in the coarser soil fractions. The research findings obtained from this study have implications for improving human health risk assessment associated with ingestion of contaminated soil and soil-originated dusts. There is a need for further division of soil particle size fractions being used for determination of bioaccessible amounts of soil-borne potentially toxic elements.

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Caption

Figure 1 Bioaccessibility of (a) Al, (b) As, (c) Cd, (d) Cr, (e) Cu, (f) Mn, (g) Ni, (h) Pb and (i) Zn in the simulated gastric environment. All values are presented as mean ± standard error (n=3) and bars with different letters indicate significantly different ($P < 0.05$)

Figure 2 Bioaccessibility of (a) Al, (b) As, (c) Cd, (d) Cr, (e) Cu, (f) Mn, (g) Ni, (h) Pb and (i) Zn in the simulated gastrointestinal environment. All values are presented as mean ± standard error (n=3) and bars with different letters indicate significantly different ($P < 0.05$).

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Particle Size Effects on Bioaccessible Amounts of Ingestible Soil-borne Toxic Elements

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Abstract

The unified BARGE method was used to examine the effects of soil particle size on the bioaccessible amounts of potentially toxic elements in multi-contaminated soils from a closed landfill site. The results show that bioaccessible As, Al, Cd, Cr, Cu, Mn, Ni, Pb and Zn increased with decreasing soil particle size and the <0.002 mm soil fraction contained much greater amounts of the bioaccessible elements, as compared to other soil fractions (0.002-0.063 mm, 0.063-0.125 mm, and 0.125-0.250 mm). As, Al and Cr had much lower bioaccessibility, as compared to the six cationic heavy metals. In contrast with other elements, As bioaccessibility tended to be higher in the gastrointestinal phase than in the gastric phase. There was a significant soil particle size effect on bioaccessibility of As and Al in the gastrointestinal phase: As bioaccessibility decreased with decreasing particle size, and the finer soil fractions tended to have a higher Al bioaccessibility, as compared to the coarser soil fractions. The research findings prompt the need for further division of soil particle size fractions in order to more accurately assess the bioaccessible amounts of soil-borne potentially toxic elements in contaminated lands.

Keywords: Soil ingestion, potentially toxic element, bioaccessibility, contaminated land, human health risk, soil particle size

1 Introduction

In vitro methods to simulate dissolution of soil-borne potentially toxic elements (SBPTEs) in human gastrointestinal tract have gained increasing acceptance as a routine protocol for assessing the human health risk from ingesting SBPTEs (Oomen et al., 2002; Li et al., 2015). The unified bioaccessibility method (UBM) developed by the Bioaccessibility Research Group of Europe (BARGE) recommended the use of <250 µm soil fraction for assessment of bioaccessibility of SBPTEs (Wragg et al., 2011; Denys et al., 2012; Collins et al., 2015). This assumes that all soil particles with a diameter less than 250 µm are proportionally ingested by human during a given period of time. However, it is highly likely that finer soil particles tend to be more ingestible, as compared to coarser soil particles. For example, Ikegami et al. (2014) found that over 90% of soil particles adhered to children's hands were <100 µm in diameter. Ingestible dust particles derived from soils could be much finer than dermally adhered soil particles (Mahowald et al., 2014). Since heavy metal concentrations tend to be higher in the finer fraction than in the coarser fraction (Li et al., 2014; Zong et al., 2016), the standard method using <250 µm soil fraction has its limitations when being used to assess human health risk associated with soil ingestion by children or ingestion of soil-derived dusts in the areas adjacent to contaminated lands (Martin et al., 2015). In addition, most of the work reported so far focused on a limited number of SBPTEs, especially lead and arsenic (Basta et al., 2007; Denys et al., 2007; Palumbo-Roe and Klinck, 2007; Bosso and Enzweiler, 2008; Denys et al., 2009; Broadway et al., 2010; Roussel et al., 2010; Lu et al., 2011; Mingot et al., 2011; Appleton et al., 2012). For soils contaminated with multiple SBPTEs, the damaging effects of ingesting such soil materials on human health could be much more severe due to combined toxicity of various SBPTEs. It is therefore important to understand the simultaneous responses of different SBPTEs to chemical attack from gastrointestinal fluids in order to better assess the combined toxic effects of multiple SBPTEs on human beings exposed to different sizes of ingestible soil/soil-derived dust particles.

In this study, four further-divided fractions of <250 μm soil fraction were used to examine the effects of soil particle size on extracting a range of potentially toxic elements from contaminated soils using the unified bioaccessibility method (UBM) in vitro gastrointestinal tract method. The objective was to determine whether further particle size division is necessary in order to more accurately assessing the human health risk from ingestion of multi-contaminated soil/soil dust particles originated from contaminated lands.

2 Materials and Methods

2.1 The soil materials

Two multi-contaminated soil materials were selected for this study following a soil screening test to identify appropriate soil samples from 27 locations within a closed landfill site adjacent to a residential area in the Greater Manchester region, England. This site received substantial amounts of industrial wastes during the period of Industrial Revolution. The soil samples were collected from the surface soil layer (0-10 cm), which is a readily available source of contaminated soil materials for human ingestion. The soil samples chosen contained a wide range of multiple SBPTEs at elevated concentrations with Sample FG 1 having a slightly alkaline pH and Sample FG2 having a slightly acidic pH (Table 1).

After collection, the soil materials were oven-dried at 40 °C and then crushed to pass a series of sieves (2.00, 0.25, 0.125 and 0.063 mm) to obtain the following soil particle fractions: 0.25-2.00 mm, 0.125-0.250 mm, 0.063-0.125 mm, and <0.063 mm. The <0.063 mm fraction was then suspended in deionized water and stirred by a magnetic stirrer for 1 h. The dispersed soil suspension was placed in a 1 L cylinder and stood for 24 h to allow the >0.002 mm soil particles to settle (McCarty et al., 2016). The remaining suspended materials were then separated from the settled soil materials to obtain two further soil particle fractions: <0.002 mm and 0.002-0.063 mm. The 0.25-2.00 mm

fraction was not used in this experiment. The four soil fractions tested in this study therefore include: <0.002 mm, 0.002-0.063 mm, 0.063-0.125 mm and 0.125-0.250 mm. The percentage share of these different soil fractions in the <0.250 mm soil mass was 1% (<0.002 mm fraction), 13% (0.002-0.063 mm fraction), 32% (0.063-0.125 mm fraction) and 54% (0.125-0.250 mm fraction) for Sample FG1, and 1% (<0.002 mm fraction), 20% (0.002-0.063 mm fraction), 32% (0.063-0.125 mm fraction) and 47% (0.125-0.250 mm fraction) for Sample FG2 (Table 1).

2.2 Element extraction procedure

The unified BARGE method (Denys et al., 2012) was slightly modified and used for extraction of a range of elements in the soil samples. Briefly, for the gastric phase extraction, 9.0 mL of synthetic saliva was added to 0.6 g of each soil sample in a 50 mL centrifuge tube. After shaking by hand for 10 seconds, 13.5 mL of synthetic gastric fluid was added to the tube. The pH of the suspension was adjusted to 1.20 ± 0.05 with HCl or NaOH. The capped tube was placed in a water bath and the content was incubated at 37 °C for 1 h. After incubation, the gastric phase sample was centrifuged for 15 min at 4500 rpm. The supernatant was then filtered, acidified with 0.5 mL concentrated HNO_3, and stored at 4 °C prior to analysis. For the gastrointestinal phase extraction, a subsample after gastric phase extraction described above was further treated with 9 mL of synthetic bile and 27 mL of synthetic duodenal fluids. The pH of the extracting solution was adjusted to 6.3 ± 0.5. The sample was then placed in a water bath and the content was incubated at 37 ºC for a further 4 h. After incubation, the gastrointestinal phase sample was centrifuged for 15 min at 4500 rpm. The supernatant was then filtered, acidified with 1.0 mL concentrated HNO_3 and stored at 4 °C prior to analysis.

2.3 Analytical methods

The total concentration of various elements in different soil particle fractions was determined by ICP-OES (Agilent Varian 720-ES) after HNO_3-HCl-H_2O_2 digestion in a microwave digester

(CEM Corporation MARS 5 Digestion Microwave System). Element concentration in the gastric and gastrointestinal phase extracts was also measured by ICP-OES. pH in various soil-fluid mixtures was measured using a calibrated pH meter (JENWAY-3510).

2.4 Statistical analysis methods

Significant difference analysis was conducted using SPSS (version 17.0). The experimental data were analyzed by one-way analysis of variance (ANOVA) and the means compared using significant difference (Duncan) method at 5% level. The relationships between the total concentration of various elements and that extracted by the simulated gastric or gastrointestinal fluids for different soil particle fractions were determined by linear regression.

2.5 Quality control and quality assurance

All the chemicals used in the experiment were of analytical grade. Ultrapure water was used throughout the entire experiment. The experiment was performed in triplicate and all the samples were run in a single batch to allow reasonable comparison.

The relative standard deviation (RSD) ranged from 4.02 to 8.72% for the gastric phase and from 4.21 to 9.15% for the intestinal phase, indicating a high level of agreement between replicates of the experiment runs. Since no appropriate certified reference materials (CRM) with comparable soil particle size fractions and the required range of contaminants are available at the time of this work, no CRM was used in this study. While the accuracy of the data was not confirmed, the high repeatability of replicated experiment ensure that the data are sufficiently precise for comparison between the different soil particle fractions. Therefore, the experimental data is of satisfactory quality for this work aiming at examining the effects of soil particle size on mobilization of SBPTEs in simulated gastrointestinal tract environments.

3 Results

3.1 Total element concentration

The total concentration of various elements estimated by HNO_3-HCl-H_2O_2 digestion method is given in Table 2. In general, there was a clear trend that the concentration of each element significantly increased with decreasing soil particle size. Except for Cr and As in FG2, the concentration of any elements for the <0.002 mm fraction was at least 1 time higher than that for the 0.002-0.063 mm fraction.

3.2 Elements by the gastric phase extraction

There was a clear trend that the amount of elements extracted by the synthetic gastric fluid increased with decreasing particle size for all the investigated elements in all the two samples (Table 3). The <0.002 mm fraction had the absolutely highest concentration in all cases with a value at least 1 time higher than that of any other soil particle fractions. In FG1, there was no significant difference in concentration between the 0.063-0.125 mm fraction and the 0.125-0.25 mm fraction for Al, Cr, Fe,Ni, Pb and Zn. In FG2, there was no significant difference in concentration between the 0.002-0.063 mm fraction and the 0.063-0.125 mm fraction for Al, As, Cu, Fe, Mn, Pb and Zn.

3.3 Elements by the gastrointestinal phase extraction

A similar trend to the gastric phase extraction was observed for the gastrointestinal phase (Table 4). The amount of elements extracted tended to increase with decreasing particle size for all the investigated elements in all the two samples. The <0.002 mm fraction had the absolutely highest concentration in all cases though the value gap between the <0.002 mm fraction and other fractions was not as large as those in the gastric phase. In FG1, there was no significant difference in concentration between the 0.063-0.125 mm fraction and the 0.125-0.25 mm fraction for the amount of elements, except for Cu and Mn. In FG2, there was no significant difference in As between the

164 0.002-0.063 mm fraction and the 0.063-0.125 mm fraction, and Cr between the 0.063-0.125 mm
165 fraction and the 0.125-0.25 mm fraction.

166

167 **4 Discussion**

168 There was a close relationship between the total concentration and either gastric phase or
169 gastrointestinal phase for all the investigated elements (Table 5). This indicates the strong control of
170 total amount of an element on the mobilizable amounts of that element in the gastrointestinal tract.
171 Therefore, the observed trend that the bioaccessible amount of either gastric phase or gastrointestinal
172 phase increased with decreasing soil particle size can be largely attributed to the increase in the total
173 element concentration with decreasing soil particle size. In terms of bioaccessibility (the percentage
174 of bioaccessible fraction in the total element pool), the particle size effect was more complex for the
175 investigated elements (Fig. 1 and Fig. 2).

176 The bioaccessibility of the investigated elements varied from element to element. For arsenic,
177 the bioaccessibility for the gastric and intestinal phases were less than 7% and 13% (Fig. 1b and Fig.
178 2b), respectively. The relatively higher bioaccesibility of As in gastrointestinal phase in spite of
179 higher pH in the extracting solution suggests that mobilization of soil-borne As in the gastrointestinal
180 tract was not largely controlled by the fluid acidity. During the gastric phase extraction, As bound to
181 iron compounds might be mobilized due to dissolution of these iron compounds under acidic
182 conditions. However, the dissolution rate of iron compounds tended to be very small with only <1%
183 of the total soil Fe being dissolved in the simulated gastric fluid for most of the soil particle fractions
184 (refer to Tables 2 and Table 3). This low solubility of iron compounds could limit the release of As
185 from the soil. During the intestinal phase extraction, the introduction of bicarbonate could enhance
186 the liberation of As from the soil under circumneutral pH conditions (Saalfield and Bostick, 2010).
187 There was no statistically significant difference in gastric phase of As among the four different

188 particle fractions. However, the bioaccessibility of the gastrointestinal As phase decreased
189 significantly with decreasing particle size, suggesting that As species held by the coarser soil
190 particles tended to be mobilized easily in the intestinal environment. This may be attributed to
191 weaker affinity between the As species and the binding site on the coarser soil particles. Li et al.
192 (2015) also observed higher As bioaccessibility for gastrointestinal phase, relative to gastric phase in
193 some As-contaminated soils.

194 Another oxyanion-forming element, Cr, also had very low bioaccessibility (<4%) in both the
195 simulated gastric and gastrointestinal environments (Fig. 1d and Fig. 2d). However, unlike As, Cr
196 bioaccessibility tended to be higher under the simulated gastric conditions than under the simulated
197 gastrointestinal conditions, suggesting that the acidity of digestive fluid plays a certain role in
198 mobilizing Cr in gastrointestinal tract. The results obtained from this study generally agreed with the
199 work by Broadway et al. (2010) who reported a Cr bioaccessibility of 5% in some Glasgow soils and
200 the bioaccessibility of Cr was greater in the gastric phase than in the gastrointestinal phase by a
201 factor of 1.5.

202 In contrast with oxyanion-forming elements, all other metals investigated in this study showed
203 much higher bioaccessibility in the simulated gastric environment (>30% except for Ni; refer to Fig.
204 1c, Fig. 1e-i, Fig. 2c and Fig. 2e-i). This suggests that most of soil-borne heavy metals were highly
205 soluble in the simulated gastric fluid. However, it is interesting to note that the bioaccessibility of
206 these metals was also very much higher in the simulated gastrointestinal environment where the fluid
207 pH was raised to 6.3. This can be attributed to the presence of digestive enzymes and bile in the
208 simulated intestinal fluid (Oomen et al., 2004; Li et al., 2013). Bile is capable of forming soluble
209 metal complexes under non-acidic pH conditions (Feroci et al., 1995; Oomen et al., 2003). Within
210 these metals, Cu exhibited an unusually high bioaccessibility (>35% for FG1 and >25% for FG2).
211 This was in agreement with work by Li et al. (2013) who found that Cu had much higher
212 bioaccessibility, as compared to other divalent heavy metals.

213 The relatively low bioaccessibility of Pb may be attributed to its low solubility under high pH
214 conditions. Close examination found that for Cd, Cu, Pb and Zn in FG1, the bioaccessibility tended
215 to be higher in the coarser fraction than in the finer fractions, suggesting that for certain soil
216 materials, a higher proportion of these metals that are bound to coarser particles may be liberated
217 from the surfaces of these soil particles.

218 Aluminium (Al) bioaccessibility ranged from 5.9 to 13% for the gastric phase. However, it
219 markedly decreased to less than 3% for the gastrointestinal phase (Fig. 1a and Fig. 2a). This reflected
220 re-immobilization of Al upon change in pH from 1.2 under gastric condition to 6.3 under intestinal
221 condition. Shock et al. (2007) investigated the bioaccessibility of Al in the soils affected by mine
222 dust deposition and found a range of 0.31−4.0% for Al bioaccessibility in the gastric phase. They
223 concluded that the low value of Al bioaccessibility in their samples was consistent with the
224 predominance of Al in relatively insoluble aluminosilicate minerals. Reis et al. (2014) also reported a
225 level of gastric phase Al bioaccessibility similar to that of Shock et al. (2007) for some French soils
226 and dusts. The relatively higher gastric phase Al bioaccessibility of soils in this study appears to
227 suggest that there was a higher proportion of more soluble Al species present in the investigated
228 soils. It is interesting to note that the gastrointestinal phase Al bioaccessibility tended to be higher in
229 the finer fractions (<0.002 mm and 0.002-0.063 mm) than in the coarser fractions (0.063-0.125 mm
230 and 0.125-0.250 mm). The reasons for this are not clear. Probably, the finer fractions contained more
231 organically complexed Al species, which could stay soluble under circumneutral pH conditions
232 (Driscoll and Letterman, 1988) such as that in the simulated intestinal environment.

233 The research findings obtained from this study have implications for assessing health risk
234 associated with human exposure to ingestible contaminated soils. For more accurate evaluation of
235 human health risk from contaminated soil/soil-originated dusts, further division of finer soil fractions
236 from the standard <0.25 mm fraction is needed. For the clay fraction (<0.002 mm), significantly
237 larger amounts of elements are mobilized in the simulated gastric and intestinal environments. This

increased amount of bioaccessible SBPTEs is particularly relevant to assessing human health risk from exposure to contaminated soils in areas experiencing frequent strong wind. Previous research demonstrated that the heavy metals could move with wind along the wind direction and the soil samples had higher heavy metal concentrations at the downwind direction (Chen et al., 2010). While Al is viewed as a potentially toxic element (Krewski et al., 2007), bioaccessibility assessment of soil-borne Al is rare. The significantly elevated gastrointestinal phase Al bioaccessibility in the finer soil fractions discovered in this study is important since bioaccessible Al tends to be primarily adsorbed in the distal intestine (Krewski et al., 2007).

5 Conclusion

The size of soil particles had significant effects on the bioaccessible elements of potential toxicity. There was a clear trend showing that As, Al, Cd, Cr, Cu, Mn, Ni, Pb and Zn increased with decreasing soil particle size and the <0.002 mm soil fraction contained much greater amounts of the bioaccessible elements, as compared to the coarser soil fractions investigated in this study. Cd, Cu, Mn, Ni, Pb and Zn tended to have much greater bioaccessibility than did As, Al and Cr. As bioaccessibility tended to be higher in the gastrointestinal phase than in the gastric phase. There was a significant soil particle size effect on bioaccessibility of As and Al in the gastrointestinal phase. As bioaccessibility decreased with decreasing particle size. But Al bioaccessibility tended to be higher in the finer soil fractions than in the coarser soil fractions. The research findings obtained from this study have implications for improving human health risk assessment associated with ingestion of contaminated soil and soil-originated dusts. There is a need for further division of soil particle size fractions being used for determination of bioaccessible amounts of soil-borne potentially toxic elements.

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Caption

Figure 1 Bioaccessibility of (a) Al, (b) As, (c) Cd, (d) Cr, (e) Cu, (f) Mn, (g) Ni, (h) Pb and (i) Zn in the simulated gastric environment. All values are presented as mean ± standard error (n=3) and bars with different letters indicate significantly different ($P < 0.05$)

Figure 2 Bioaccessibility of (a) Al, (b) As, (c) Cd, (d) Cr, (e) Cu, (f) Mn, (g) Ni, (h) Pb and (i) Zn in the simulated gastrointestinal environment. All values are presented as mean ± standard error (n=3) and bars with different letters indicate significantly different ($P < 0.05$).

Figure 1

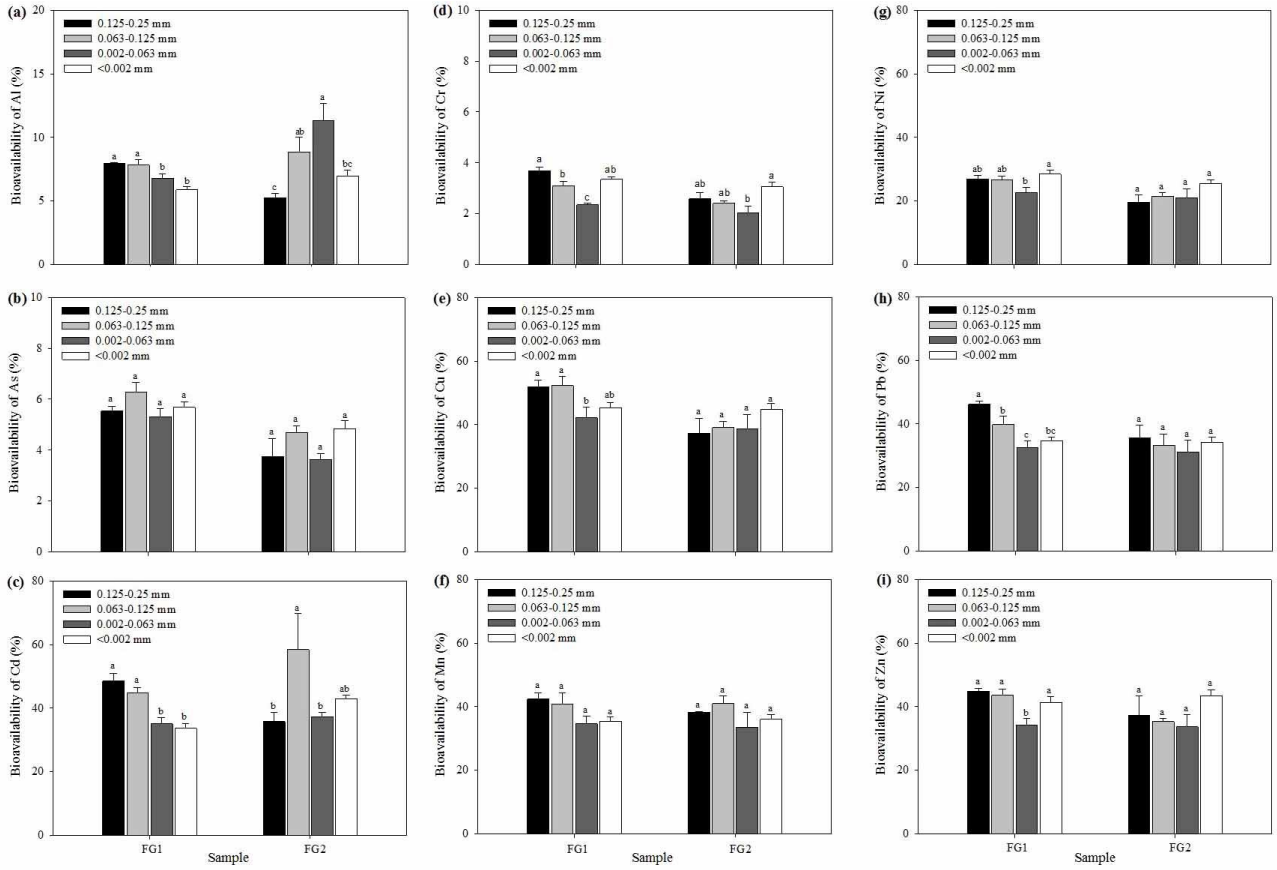

Figure 1

Figure 2

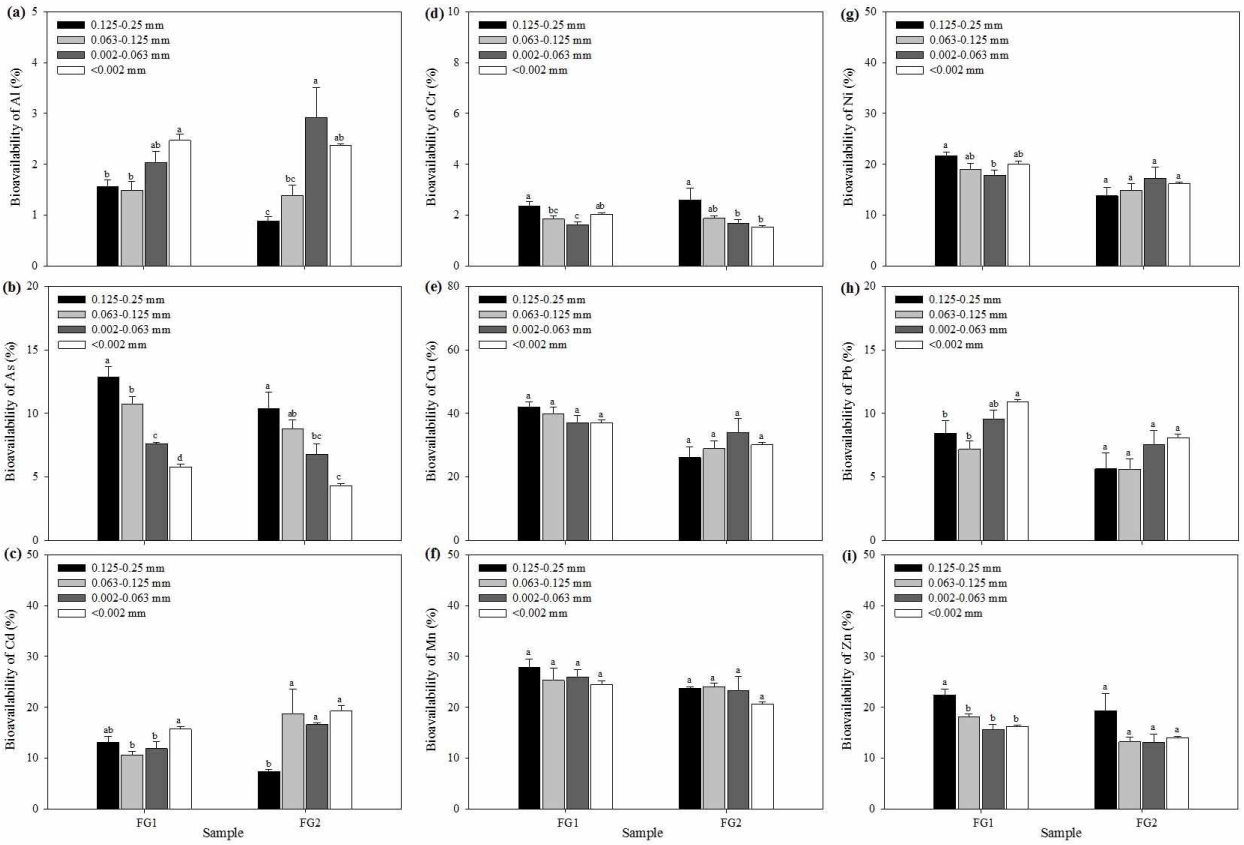

Figure 2

Table 1
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Table 1 Some major physical and chemical characteristics of the soil materials used in this study

Soil parameter	FG1	FG2
pH	7.12	6.62
EC (dS/m)	0.039	0.035
Organic carbon content (%)	1.11	1.27
0.125-0.25 mm fraction (%)	54	47
0.063-0.125 mm fraction (%)	32	32
0.002-0.125 mm fraction (%)	13	20
<0.002 mm fraction (%)	1	1
Total As (mg/kg)	29.6	27.2
Total Al (mg/kg)	21013	19247
Total Ca (mg/kg)	32166	32173
Total Cd (mg/kg)	6.19	9.28
Total Cr (mg/kg)	68.8	68.8
Total Cu (mg/kg)	2768	3377
Total Fe (mg/kg)	28131	21833
Total K (mg/kg)	1859	2423
Total Mn (mg/kg)	3865	3511
Total Na (mg/kg)	959	799
Total Ni (mg/kg)	811	925
Total Pb (mg/kg)	1498	1597
Total Zn (mg/kg)	1276	1031

Table 2
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Table 2 Total concentration of various elements estimated by HNO_3-HCl-H_2O_2 digestion

	Particle fraction (mm)			
Element (mg/kg)	0.125-0.25	0.063-0.125	0.063-0.002	<0.002
Sample FG1				
Al	8640±91.4c	10244±294c	13898±614b	44247±842a
As	11.3±0.35d	15.6±0.50c	25.3±0.47b	60.7±0.46a
Cd	1.14±0.06d	1.64±0.04c	2.53±0.10b	7.25±0.12a
Cr	30.1±0.50d	42.2±1.38c	65.2±2.28b	103±0.94a
Cu	957±39.0d	1281±27.8c	2077±62.8b	5846±65.4a
Fe	22129±1151d	28446±539c	39839±694b	47691±791a
Mn	925±47.7d	1249±78.8c	1773±43.0b	3805±46.1a
Ni	295±10.8d	395±12.6c	596±12.6b	1509±26.0a
Pb	484±11.5d	673±17.3c	1132±23.0b	2683±40.7a
Zn	352±6.62d	471±9.77c	749±14.2b	2197±31.5a
Sample FG2				
Al	8423±175c	11552±243b	12612±229b	47375±1132a
As	12.5±1.74d	19.2±1.89c	29.7±1.91b	55.6±0.59a
Cd	2.08±0.20c	2.19±0.44c	4.51±0.03b	10.5±0.50a
Cr	30.1±4.13d	44.5±3.07c	61.3±6.45b	93.9±0.76a
Cu	826±8.96d	1061±40.2c	1393±28.2b	3429±27.8a
Fe	23526±2148c	30084±1612b	29328±1143b	48341±349a
Mn	771±20.0c	1126±40.4c	1764±237b	3740±33.5a
Ni	309±38.2c	453±41.6c	614±70.2b	1422±11.6a
Pb	323±42.6d	524±60.2c	741±87.9b	1706±11.8a
Zn	321±46.5c	508±19.4b	643±71.9b	1811±14.4a

All values are presented as mean ± standard error (n=3). Means with different letters in the same row are significantly different at $P < 0.05$.

Table 3
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Table 3 Concentration of various elements by the gastric phase extraction

	Particle fraction (mm)			
Element (mg/kg)	0.125-0.25	0.063-0.125	0.063-0.002	<0.002
Sample FG1				
Al	684±2.52c	798±23.2c	941±49.1b	2592±59.7a
As	0.63±0.03d	0.98±0.06c	1.34±0.06b	3.45±0.14a
Cd	0.55±0.00c	0.73±0.01b	0.88±0.03b	2.43±0.10a
Cr	1.10±0.03c	1.30±0.03c	1.53±0.10b	3.48±0.08a
Cu	496±2.27d	668±21.0c	870±42.9b	2649±78.9a
Fe	116±2.90c	134±3.84bc	159±13.4b	426±8.29a
Mn	390±1.57d	506±12.0c	611±27.6b	1345±41.2a
Ni	79.4±0.38c	104±2.22c	135±7.12b	428±14.1a
Pb	223±1.00c	266±10.6c	367±18.6b	932±20.8a
Zn	158±0.59c	204±5.36bc	256±10.8b	908±37.1a
Sample FG2				
Al	606±23.5c	896±65.6bc	1072±16.5b	3296±166a
As	0.44±0.02c	0.89±0.05b	1.07±0.03b	2.68±0.17a
Cd	0.73±0.01d	1.18±0.03c	1.68±0.06b	4.51±0.24a
Cr	0.76±0.04c	1.06±0.07bc	1.22±0.01b	2.88±0.18a
Cu	262±5.09c	412±21.8b	526±4.75b	1537±74.5a
Fe	96.3±6.64c	184±14.4b	229±14.7b	529±30.6a
Mn	295±9.12c	461±28.1b	568±13.9b	1348±57.6a
Ni	58.9±2.00d	96.2±4.04c	126±1.22b	364±16.9a
Pb	112±7.61c	171±12.5b	224±2.00b	582±29.0a
Zn	114±0.56c	180±7.16b	210±1.11b	785±37.3a

All values are presented as mean ± standard error (n=3). Means with different letters in the same row are significantly different at $P < 0.05$.

Table 4
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Table 4 Concentration of various elements by the gastrointestinal phase extraction

	Particle fraction (mm)			
Element (mg/kg)	0.125-0.25	0.063-0.125	0.063-0.002	<0.002
Sample FG1				
Al	135±13.8c	151±12.9c	281±18.2b	1092±30.2a
As	1.45±0.04c	1.66±0.05bc	1.92±0.06b	3.51±0.14a
Cd	0.15±0.01c	0.17±0.01c	0.30±0.03b	1.14±0.03a
Cr	0.71±0.06c	0.78±0.03c	1.06±0.03b	2.11±0.07a
Cu	401±2.38d	510±14.1c	767±35.5b	2164±37.7a
Fe	41.6±3.19c	45.6±5.46c	63.8±5.56b	210±3.73a
Mn	256±7.31d	313±14.0c	460±15.6b	932±17.4a
Ni	63.8±0.66c	74.7±2.58c	106±4.37b	301±5.32a
Pb	40.7±4.13c	47.9±3.35c	108±7.51b	292±2.71a
Zn	78.7±3.35c	85.0±1.19c	117±6.69b	357±5.98a
Sample FG2				
Al	102±11.0c	157±8.34c	273±27.6b	1127±38.5a
As	1.25±0.07c	1.66±0.06b	1.98±0.16b	2.40±0.08a
Cd	0.15±0.02d	0.37±0.02c	0.75±0.01b	2.02±0.04a
Cr	0.75±0.02c	0.83±0.02c	1.01±0.05b	1.45±0.04a
Cu	183±2.58d	302±5.44c	460±18.4b	1034±17.7a
Fe	36.7±3.19c	52.9±2.88bc	77.7±8.04b	185±14.3a
Mn	183±7.04d	270±4.45c	396±1.75b	774±12.0a
Ni	41.3±0.87d	66.4±1.15c	103±2.88b	229±4.45a
Pb	17.2±1.75d	28.4±1.65c	53.9±4.06b	138±4.58a
Zn	58.8±1.19d	66.8±2.31c	82.0±0.87b	254±3.19a

All values are presented as mean ± standard error (n=3). Means with different letters in the same row are significantly different at $P < 0.05$.

Table 5
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Table 5 Linear relationship between the total concentration (Y) and gastric phase or gastro-intestinal phase (X) for each element in different soil particle fractions

Soil sample	Elements	Regression equations	R	*p*-value
FG1	Gastric phase			
	Al	y = 18.57x – 4023.48	0.994	0.000
	As	y = 17.47x + 0.31	0.993	0.000
	Cd	y = 3.19x – 0.53	0.991	0.000
	Cr	y = 27.99x + 8.46	0.948	0.000
	Cu	y = 2.34x – 81.56	0.993	0.000
	Mn	y = 2.95x – 167.19	0.987	0.000
	Ni	y = 3.37x + 70.24	0.989	0.000
	Pb	y = 3.00x – 99.08	0.990	0.000
	Zn	y = 2.38x + 32.71	0.991	0.000
	Gastrointestinal phase			
	Al	y = 36.53x + 4103.54	0.992	0.000
	As	y = 23.40x – 21.76	0.986	0.000
	Cd	y = 5.91x + 0.54	0.993	0.000
	Cr	y = 47.81x + 4.63	0.959	0.000
	Cu	y = 2.75x – 98.32	0.997	0.000
	Mn	y = 4.18x – 108.98	0.992	0.000
	Ni	y = 4.95x + 23.21	0.994	0.000
	Pb	y = 8.46x + 209.45	0.995	0.000
	Zn	y = 6.39x – 75.29	0.995	0.000
FG2	Gastric phase			
	Al	y = 14.73x – 1876.45	0.988	0.000
	As	y = 18.78x + 5.36	0.969	0.000
	Cd	y = 2.30x + 0.15	0.986	0.000
	Cr	y = 27.12x + 17.32	0.928	0.000
	Cu	y = 2.03x + 284.19	0.995	0.000
	Mn	y = 2.78x – 5.58	0.975	0.000
	Ni	y = 3.58x + 123.61	0.984	0.000
	Pb	y = 2.85x + 47.77	0.980	0.000
	Zn	y = 2.14x + 131.61	0.988	0.000
	Gastrointestinal phase			
	Al	y = 37.86x + 4343.12	0.996	0.000
	As	y = 33.35x – 31.56	0.900	0.000
	Cd	y = 4.70x + 0.96	0.981	0.000
	Cr	y = 84.72x – 28.00	0.958	0.000
	Cu	y = 3.12x + 129.90	0.991	0.000
	Mn	y = 5.08x – 212.62	0.988	0.000
	Ni	y = 5.93x + 47.25	0.986	0.000
	Pb	y = 11.04x + 168.61	0.976	0.000
	Zn	y = 7.207x – 9.24	0.987	0.000